APPLICATION NOTE

BioQule DNA-Seq library prep: Samples to ready-to-use NGS libraries.

Introduction

The BioQule™ NGS system is an innovative automated platform that simplifies commercially available Next Generation Sequencing workflows with the push of a button. This makes Next Generation Sequencing (NGS) readily accessible, with automated preparation of sequencing ready libraries.
This offers a reliable and user-friendly complete system for automated low throughput nucleic acid library preparation. Revvity's BioQule NGS System can fully automate library preparation for up to 8 samples in a single workflow, reducing the risk of human error and minimizing hands-on time to only 15 minutes. This fully walk-away sample preparation and quantification benchtop instrument comes at low-cost for automating library preparation from human genomic DNA.
The results in this application note are focused on utilizing the BioQule DNA Library Prep Kit, which utilizes enzymatic fragmentation, and the BioQule NGS System for Whole Genome Sequencing (WGS).

Methods

The BioQule Cartridge (Revvity, PN. CLS158240) and BioQule DNA-Seq Library Prep Kit (P/N 900-000020) were used for library preparation. BioQule DNA-Seq Library Prep Kit is pre-plated, and the user needs to manually add minimum reagents. A total of 24 samples of human genomic.

A total of 24 samples of human genomic DNA samples were utilized for library preparation. The genomic DNA input amount in each sample was 40 ng in a total volume of 10 µL. The input range for the BioQule -DNA Seq assay is 20 ng to 500 ng.

The library preparation was done using the BioQule DNA-Seq assay and the NEXTFLEX™ unique Dual Index Barcodes with 6 PCR cycles. The LabChip™ GX Touch™ microfluidic electrophoresis platform was the employed to assess the size range and yield of the library outputs.

The BioQule NGS system results (Automation workflow) were compared to the Manual workflow in Figure 1 and Figure 2. Sequencing of both the automated and manually prepared samples was performed using the MiniSeq® platform at 2x150 (Illumina, San Diego, CA).

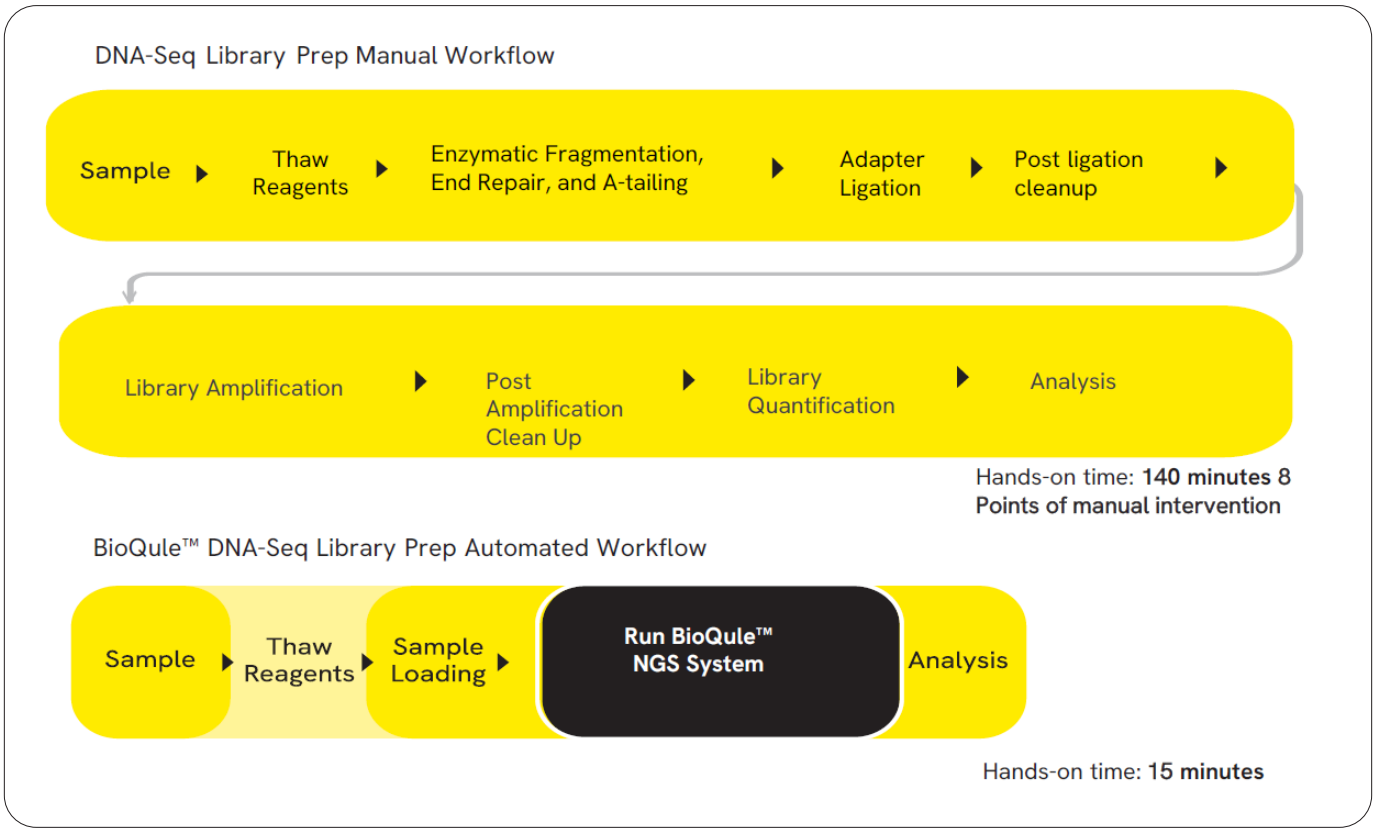

Figure 1: Manual DNA-Seq Library Prep Workflow vs BioQule DNA-Seq Library Prep Automated workflow for nucleic acid library preparation and quantification.

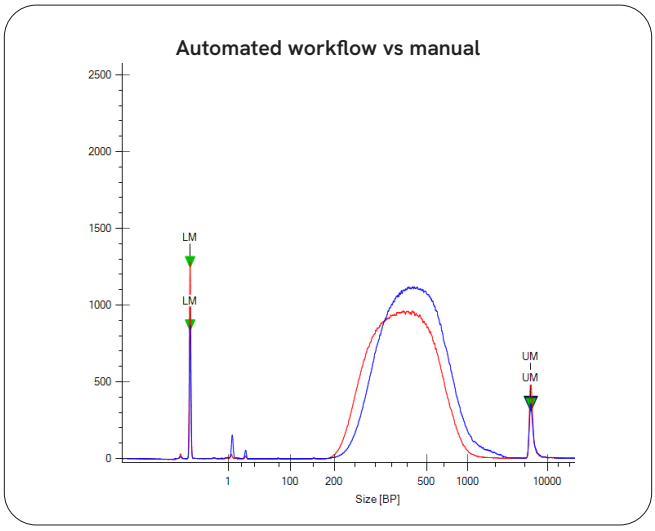

Figure 2: Comparison of electropherogram traces from libraries prepared using the BioQule NGS system (red) or Manual workflow (blue).

The quality of the libraries was evaluated by looking at the insert sizes. Comparison was the extended to the analysis of the GC bias. The results (Figure 3) show comparable insert sizes between the manual and the BioQule workflow. The data showed consistent distribution of insert sizes, which was also comparable between the two workflows. There was limited variance (Figure 4) in both the manual DNA-Seq Library Prep workflow and the BioQule DNA-Seq Library Prep Automated workflow when the GC bias was analyzed across the 20 – 60 % GC windows (~95% of the human genome). Both workflows also showed minimal variability in normalized coverage across this window.

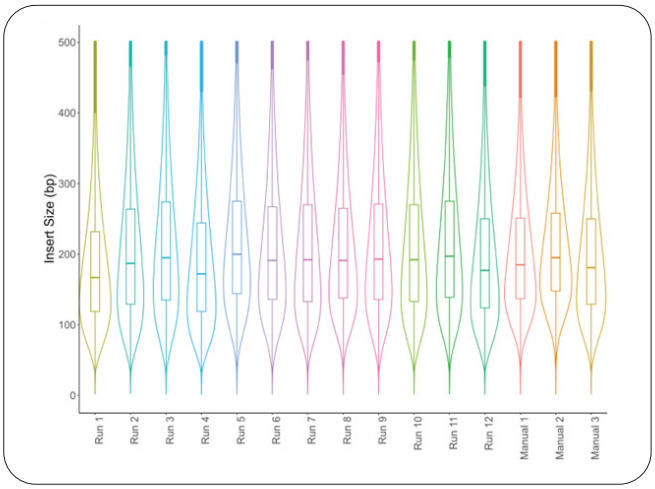

Figure 3: Insert sizes for 12 libraries prepared on the BioQule NGS System and 3 libraries prepared manually, grouped by run. Data shows consistent distribution of insert sizes, with BioQule libraries comparable to manual runs.

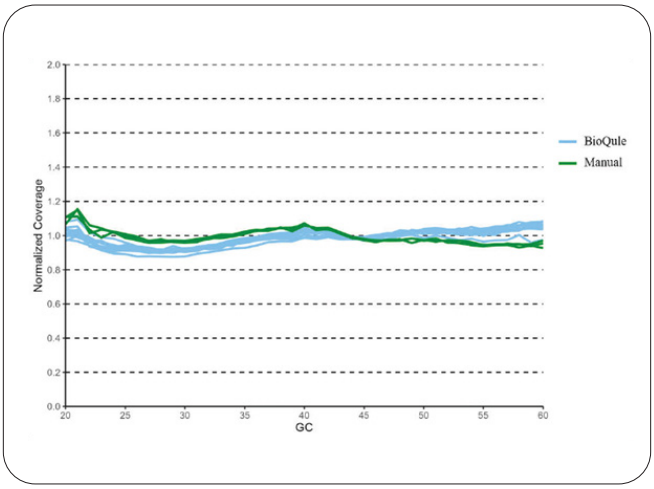

Figure 4: Analysis of GC bias from 15 libraries prepared with the BioQule NGS System (blue) vs manual workflow (green). The results show minimal variability in normalized coverage across 20-60% GC (~95% of the human genome).

Conclusion

The BioQule NGS System can fully automate the preparation of consistent sequencing-ready libraries from human genomic DNA samples. The pre-plated BioQule DNA-Seq Library Kit (P/N 900-000020), which has integrated enzymatic fragmentation, can be used on the BioQule NGS system to produce up to 8 libraries in a single run with size range, yield, insert size and GC bias comparable to the manual workflow. The BioQule NGS system offers a reduction in hands-on time, elevating laboratory efficiency, while minimizing human errors. This automated workflow offers library preparation and quantification from human Genomic DNA to NGS sequencing ready libraries accessible to laboratories of all sizes and technical expertise.